

Organochlorines in Breast Milk from Two Cities in Ukraine

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Reports of environmental problems in the former Soviet Union, including excess use of pesticides, have led to concerns about high levels of contamination in humans, but little information is available to assess whether these concerns are warranted. Samples of breast milk from 197 women from two cities in Ukraine were analyzed for *p,p'*-DDT, *p,p'*-DDE, endrin, dieldrin, heptachlor epoxide, *trans*-nonachlor, oxychlordane, hexachlorobenzene, β -hexachlorocyclohexane (HCH), and 18 polychlorinated biphenyl congeners, and results were compared to previous reports from Europe. The median β -HCH concentration was 731 ng/g milk fat, which is higher than other reports from Europe but lower than reports from other parts of the world. The median DDE concentration was 2,457 ng/g milk fat, which is higher than most but not all other reports from Europe. Concentrations of other chemicals were comparable to or lower than other reports from Europe. Concentrations from the city of Kyiv were generally lower than those from Dniprodzerzhinsk, but the magnitudes of these differences were modest. **Key words:** DDE, DDT, dieldrin, endrin, heptachlor epoxide, hexachlorobenzene, hexachlorocyclohexane, milk, oxychlordane, polychlorinated biphenyls, *trans*-nonachlor. *Environ Health Perspect* 107:459–462 (1999).

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Materials and Methods

The subjects were chosen from among the participants in a study of reproductive and child health being carried out at several sites in Ukraine (1). Subjects in this study were pregnant women in specific geographic regions during specific time periods; exclusions were made primarily to eliminate transients. Biological samples were collected at one of the study sites in Kyiv and at the site in Dniprodzerzhinsk. Kyiv is the capital of Ukraine and is the largest city, with a population of 2.6 million. Dniprodzerzhinsk is a highly industrialized city of 0.3 million. Participants at these sites who gave birth from November 1993 to December 1994 were asked to supply samples of breast milk, placenta, and cord; informed consent was obtained from all women before sample collection. Milk samples were collected 4 days after birth (or on the fifth day if, for any reason, milk could not be obtained on the fourth). After feeding the child, the mother was asked to express 5 mL of milk.

For this analysis, 100 women from each city were chosen from those who supplied all requested samples and who did not deliver twins. Women were chosen at random except for age; one-half were chosen to be under 30 years of age and one-half to be older. Breast milk samples from three women were lost during extraction. Thus, the results presented here are for 197 women, 99 from Kyiv and 98 from Dniprodzerzhinsk. Thirty-three

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The presence of a variety of organochlorine contaminants in breast milk has been reported over many years from numerous parts of the world (1); most women whose milk has been examined show at least some degree of contamination. The organochlorines found in milk include a variety of pesticides and various industrial chemicals such as polychlorinated biphenyls (PCBs). Many of these compounds bioaccumulate and have long half-lives in humans. They have been shown to have a wide variety of toxic actions, although not all have been shown to have significance for human health (1–3). The examination of breast milk can be used to indicate the general level of contamination, and thus of potential health risk, in the population. Such examinations can also be used more specifically to examine the possible risks to infants, either from transplacental exposure, which occurs during the sensitive prenatal period, or from consumption of breast milk, which transfers large quantities of chemical from mother to child.

Serious concerns have been voiced about environmental problems in the former Soviet Union. One of many specific concerns that has been raised is careless use of pesticides, with reports of inappropriate and excess use (4,5). Excess use could result in unusually severe contamination of the environment. This in turn could produce unusual contamination among the general population and, in particular, high concentrations of pesticides in human milk.

However, there are few reports available to assess whether this has, in fact, occurred. From Ukraine, reports on organochlorine contaminants in breast milk are limited to information on levels of DDT in the 1960s and 1970s (6–10).

We studied concentrations of a number of organochlorine pesticides or their persistent metabolites and of PCBs in breast milk from 197 women from two cities in Ukraine to determine whether they were comparable to those seen elsewhere in Europe. We measured *p,p'*-DDT (dichlorodiphenyltrichloroethane), *p,p'*-DDE (dichlorodiphenyldichloroethylene), endrin, dieldrin, oxychlordane, *trans*-nonachlor, heptachlor epoxide, hexachlorobenzene (HCB), β -hexachlorocyclohexane (HCH), and 18 PCB congeners. *p,p'*-DDT is the primary component of the insecticide DDT; *p,p'*-DDE is its stable degradation product. Endrin and dieldrin are insecticides; dieldrin is also the persistent metabolite of the insecticide aldrin. Oxychlordane is the persistent metabolite of the insecticide chlordane. *trans*-Nonachlor is a persistent impurity of chlordane. Heptachlor epoxide is the persistent metabolite of the insecticide heptachlor and is also an impurity in chlordane. HCB is a fungicide, and β -HCH is the persistent isomer of the lindane family of insecticides. PCBs are a family of industrial compounds with a variety of uses, including use as dielectric fluids in transformers and capacitors.

Table 1. Concentrations of organochlorine compounds (ng/g milk fat).

Organochlorine compound	Percent < MDL	Median	90th percentile	Range
Pesticides				
<i>p,p'</i> -DDE	0	2,457	5,663	328–17,412
β -HCH	0	731	1,305	137–2,387
<i>p,p'</i> -DDT	1	336	735	< 26–3,150
HCB	2	168	287	< 18–1,297
<i>trans</i> -Nonachlor	16	20	37	< 12–73
Oxychlorodane	27	18	35	< 13–82
Heptachlor epoxide	39	16	37	< 14–244
Endrin	81	< 17	28	< 17–304
Dieldrin	99	< 18	< 18	< 18–38
PCB congeners ^a				
153/132	0	149	292	27–1,525
138/160	0.5	134	244	< 22–1,252
118	0.5	93	168	< 13–408
170/190	1	72	163	< 17–472
180	0.5	55	128	< 15–903
66	5	24	45	< 12–128
101/90	23	23	43	< 17–94
105	8	18	37	< 8–90
28	48	14	30	< 14–127
187	45	12	33	< 12–288
44	68	< 17	31	< 17–75
52	73	< 18	26	< 18–76
8/5	83	< 17	71	< 17–307
18/17	95	< 13	< 13	< 13–48
128	95	< 13	< 13	< 13–46
209	97	< 13	< 13	< 13–81
195/208	98	< 13	< 13	< 13–36
206	100	< 13	< 13	< 13–11

Abbreviations: HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; MDL, method detection limit; PCB, polychlorinated biphenyl.

^aInternational Union of Pure and Applied Chemistry numbers.

Table 2. Organochlorine concentrations by city (ng/g milk fat).

Organochlorine compound	Kyiv		Dniprodzerzhinsk		Significance ^a
	Median	Percent < MDL	Median	Percent < MDL	
Pesticides					
<i>p,p'</i> -DDE	2,752	0	2,254	0	NS
β -HCH	753	0	714	0	NS
<i>p,p'</i> -DDT	345	2	322	0	NS
HCB	151	0	186	3	$p < 0.01$
<i>trans</i> -Nonachlor	16	25	23	7	$p < 0.01$
Oxychlorodane	16	33	22	21	$p < 0.01$
Heptachlor epoxide	< 14	61	22	17	$p < 0.01$
Endrin	< 17	69	< 17	94	$p < 0.01$
Dieldrin	< 18	99	< 18	100	NS
PCB congeners ^b					
153/32	134	0	167	0	$p < 0.01$
138/160	116	1	149	0	$p < 0.01$
118	76	1	110	0	$p < 0.01$
170/190	66	2	80	0	$p = 0.02$
180	46	1	67	0	$p < 0.01$
66	21	7	26	3	NS
101/90	19	35	27	11	$p < 0.01$
105	14	14	21	2	$p < 0.01$
28	< 14	54	15	42	NS
187	< 12	55	14	35	$p < 0.01$
44	< 17	75	< 17	60	$p = 0.03$
52	< 18	82	< 18	64	$p < 0.01$
8/5	< 17	85	< 17	81	NS
18/17	< 13	95	< 13	95	NS
128	< 13	94	< 13	97	NS
209	< 13	98	< 13	96	NS
195/208	< 13	98	< 13	99	NS
206	< 13	100	< 13	100	NS

Abbreviations: HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; MDL, method detection limit; NS, not significant; PCB, polychlorinated biphenyls.

^aSignificance level of test comparing cities, determined by Wilcoxon or Fisher's exact test (see "Materials and Methods"). ^bInternational Union of Pure and Applied Chemistry numbers.

percent of the older and 81% of the younger women were primiparous. Twenty-two percent of the older and 31% of the younger women had ever smoked, but only 1% of the older and 8% of the younger women were current smokers.

Samples were analyzed by high resolution capillary gas chromatography using electron capture detection (12). Approximately 3 g of milk (wet weight) was used for the analysis. After the addition of the surrogate standards (4,4'-dibromooctafluorobiphenyl, PCB 103, and PCB 198) and anhydrous sodium sulfate, the milk sample was extracted three times with methylene chloride using a Tissumizer homogenizer (PRO Scientific, Inc., Monroe, CT). A subsample was removed from the total volume and concentrated for determination of lipid content; the samples averaged 2.5% lipid. The combined extracts were concentrated in hexane and then purified by silica:alumina column chromatography. This fraction was further purified by HPLC to remove interfering lipids. The HPLC fraction was then concentrated to 0.5 mL in hexane and tetrachloro-*meta*-xylene was added as the internal standard.

Selected chlorinated pesticides and PCBs were analyzed by fused-silica capillary column gas chromatography with electron capture detection (Ni^{63}) in splitless mode. Temperature of the capillary column, 30 m long \times 0.25 mm inner diameter with 0.25 μm DB-5 film thickness, was programmed from 100 to 140°C at 5°C/min, from 140 to 250°C at 1.5°C/min, and from 250 to 300°C at 10°C/min, with 1 min hold time at the beginning of the program and before each program rate change. The final temperature was held for 5 min. Injector and detector temperatures were 275 and 325°C, respectively. Calibration of the detector was accomplished during the analytical sequence using four calibration mixtures, in a nonsequential order, interspersed with the samples. Analyte calculations were based on this calibration curve and reported as nanograms per gram of lipid.

For chemicals for which at least 50% of samples had levels above the method detection limit (MDL), comparisons between cities and age groups were made by Wilcoxon's signed rank test. For the remainder, the percent above the MDL was compared by Fisher's exact test. A cutoff of 0.05 (two-sided) was used for statistical significance. Correlations presented are Spearman rank correlations. Statistics presented are based on all samples.

Results

The concentrations of organochlorine pesticides found in the samples are shown in

Table 1. All of these chemicals were found in at least some of the samples. *p,p'*-DDE was present at the highest concentrations, followed by β -HCH. Table 1 also shows the concentrations of the various congeners of PCBs. Congeners 153/132 and 138/160 (International Union of Pure and Applied Chemistry numbers) were present at the highest concentrations. The distribution of most of the chemicals was skewed to the right.

The median concentrations are shown for the two cities separately in Table 2; the percent below the MDL is also shown. Differences between the two cities are evident. HCB, *trans*-nonachlor, oxychlorane, and heptachlor epoxide were all statistically significantly higher in Dniprodzerzhinsk than in Kyiv, as were many of the PCB congeners. Endrin, conversely, was significantly more likely to be detected in Kyiv.

Older women tended to have higher levels than did younger women (Table 3). The difference was statistically significant for *p,p'*-DDE, β -HCH, HCB, *trans*-nonachlor, oxychlorane, and several PCB congeners. The differences are larger if only primiparous women are included.

As expected, the pesticides do not occur independently. *p,p'*-DDE and *p,p'*-DDT have a correlation of 0.71. The median ratio of the more persistent *p,p'*-DDE to the less persistent *p,p'*-DDT is 7.5; this is typical of people with little recent exposure to the parent compound. Heptachlor epoxide, *trans*-nonachlor, and oxychlorane, all of which are components of technical chlordane, have correlations with each other of 0.64–0.67. Other correlations among pesticides are < 0.6. There are also high correlations among some of the PCBs (Table 4). All correlations between pesticides and PCBs are < 0.6.

Discussion

The β -HCH concentrations seen in this study, with a median of 731 ng/g milk fat, are high compared to those seen in other reports from Europe. Recent large studies, for example, reported β -HCH concentrations (in nanograms per gram of milk fat) of 64 in 1990 and 56 in 1991 from Germany (13); a median of 60 from the United Kingdom (14); and a median of 80 from the Netherlands (15). Hernández et al. (16) reported a mean of 235 ng/g milk fat; this is among the highest previously reported from Europe. Whereas the concentrations in the present study are higher than those usually reported from Europe, they are comparable to or lower than levels seen in some other parts of the world. They are comparable to reported concentrations (nanograms per gram of milk fat) from the

Table 3. Organochlorine concentrations by age of mother (ng/g milk fat).

Organochlorine compound	Age < 30 years		Age \geq 30 years		Significance ^a
	Median	Percent < MDL	Median	Percent < MDL	
Pesticides					
<i>p,p'</i> -DDE	2,141	0	2,752	0	$p < 0.01$
β -HCH	646	0	825	0	$p < 0.01$
<i>p,p'</i> -DDT	322	2	348	0	NS
HCB	153	2	187	1	$p < 0.01$
<i>trans</i> -Nonachlor	17	22	21	10	$p < 0.01$
Oxychlorane	16	32	20	23	$p < 0.01$
Heptachlor epoxide	15	41	17	37	NS
Endrin	< 17	83	< 17	79	NS
Dieldrin	< 18	100	< 18	99	NS
PCB congeners ^b					
153/132	134	0	166	0	$p < 0.01$
138/160	121	1	148	0	$p < 0.01$
118	82	1	98	0	$p = 0.02$
170/190	71	0	72	2	NS
180	50	1	59	0	$p = 0.02$
66	23	4	24	7	NS
101/90	23	26	23	21	NS
105	17	8	19	8	NS
28	14	49	15	46	NS
187	< 12	56	14	33	$p < 0.01$
44	< 17	63	< 17	72	NS
52	< 18	78	< 18	68	NS
8/5	< 17	82	< 17	84	NS
18/17	< 13	95	< 13	95	NS
128	< 13	94	< 13	97	NS
209	< 13	99	< 13	95	NS
195/208	< 13	98	< 13	99	NS
206	< 13	100	< 13	100	NS

Abbreviations: HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; MDL, method detection limit; NS, not significant; PCB, polychlorinated biphenyl.

^aSignificance level of test comparing age groups, determined by Wilcoxon or Fisher's exact test (see "Materials and Methods"). ^bInternational Union of Pure and Applied Chemistry numbers.

Table 4. Correlations among polychlorinated biphenyl congeners.^a

	153/132	138/160	118	180	105	187
153/132	1					
138/160	0.97	1				
118	0.84	0.87	1			
180	0.86	0.85	0.70	1		
105	0.71	0.76	0.90	0.61	1	
187	0.80	0.74	0.58	0.78	0.48	1

For congeners not shown (8/5, 18/17, 28, 44, 52, 66, 101/90, 128, 170/190, 195/208, 206, 209), all correlations are < 0.6 in absolute value.

^aInternational Union of Pure and Applied Chemistry numbers.

following: a mean of 553 from Australia at the first sampling (17), a mean of 561 from Mexico (18), and a mean of 840 from Zimbabwe (19). They are lower than, for example, the mean of 2,210 ng/g milk fat in a report from Kazakhstan (20) and the mean of 8,830 in a report from India (21).

The DDE and DDT concentrations in the present study, with medians of 2,457 and 336 ng/g milk fat, respectively, are higher than those in most, but not all, other European reports. Most large studies report lower levels. For example, in recent large studies of DDE, reported concentrations (in nanograms per gram of milk fat) were 534 and 504 in 1990 and 1991, respectively, from Germany (13); a median of 300 from the United Kingdom (14); a concentration of

approximately 500 from Sweden (22); and a median of 550 from the Netherlands (15). Czaja et al. (23) reported concentrations somewhat higher than these in Poland, with means of 0.0254 and 0.0275 mg/L in more and less industrialized areas, respectively; these concentrations correspond to about 1,000 ng/g fat at our mean of 2.5% fat. Smaller studies with relatively high concentrations (nanograms per gram of milk fat) include a report from Yugoslavia (medians of 550 and 1,080) (24), one from three regions of the Czech Republic (concentrations of 832, 998, and 1,283) (25), one from Spain (a concentration of 1,430 at the earlier sampling) (26), one from Italy (mean of 2,200) (27), and one from Poland (geometric mean of 4,180) (28).

For other pesticides, concentrations seen here are comparable to or lower than those seen in other reports from Europe. HCB concentrations reported from Europe are quite variable. Concentrations of HCB in the present study are within the reported range. They are comparable to those seen, for example, in reports from Germany (13) and Italy (27), and they are much lower than those seen in a report from Spain (29). There are fewer reports of concentrations of *trans*-nonachlor and oxychlordane from Europe. Recent results reported from Sweden (22) and Norway (30) are similar to those seen here. Results from Finland (31,32) are lower. Heptachlor epoxide levels in the present study are similar to or lower than those seen in recent large studies from the Netherlands (15) and Germany (33,34). Dieldrin levels seen here are similar to or lower than those seen in the Netherlands (15), Germany (13), and the United Kingdom (14). Endrin is seen in a minority of samples in this study, which is similar to most other reports (1).

Comparing concentrations of PCBs across studies is complicated because there are many congeners, and the congeners reported are not always the same. Congeners 153 and 138, which usually have the highest levels, are reported in many studies. Our results are within the range reported from Europe; they are comparable to those seen in reports from Sweden (22) and the Netherlands (35). Fürst et al. (13) from Germany reported higher concentrations, and Dwarka et al. (14) from the United Kingdom reported lower concentrations. Two pooled samples from Kyiv, analyzed as part of a World Health Organization survey, have values below the medians but within the ranges in the current study (36).

We have shown some differences between the concentrations of milk contamination in the two cities. However, the magnitude of these differences were generally modest. *trans*-Nonachlor, oxychlordane, and several PCBs were about 40% higher. Compared to the differences seen from one country to another, the size of the differences between the cities is small. We have also shown an increase in concentrations with the age of the mother; this has been previously reported (1) and is due to the long half-lives of these chemicals.

We expected that there might be unusual contamination of breast milk in Ukraine because of reported excess use of pesticides in the past. β -HCH and DDE/DDT show levels that are higher than those reported from other European countries, and other chemicals measured

here do not. The consequences of this contamination for infants are not clear. Follow-up of the children born to the women in this study is ongoing.

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